

were I disposed to admit into it those forms which I have detected, after a careful examination, sparingly distributed throughout the material when finally prepared for mounting. But I am reluctant to put forward as new species, or even as well-established varieties, forms whose characters rest upon what my knowledge of these organisms obliges me to regard as insufficient and unsatisfactory evidence. Not to dwell upon the liability to accidental intermixture to which such materials are liable, which, as every practical microscopist is aware, defy his most careful attention, and frequently intrude upon a gathering from one locality stray frustules belonging to a very different habitat, I consider it impossible to decide upon the specific claims of any but the most conspicuously marked Diatomaceous forms, from an examination of a few isolated specimens. In such genera as *Cymbella*, *Navicula*, *Pinnularia*, *Cocconema*, and others, where there exists great simplicity of outline combined with great diversity of size, a knowledge of the form under examination, from different localities and at different periods of development, is absolutely necessary to enable the observer to determine its specific character, and to warrant him in referring it to a known, or erecting it into a new, species. Better to leave its claims *sub lite* until more satisfactory evidence is afforded, than to introduce confusion into the nomenclature of the science, and uncertainty into our conclusions with regard to geographical distribution, by a hasty, insufficient, and temporary determination. The announcement of a new species is sometimes nothing more than the publication of the observer's imperfect knowledge: the forbearance which foregoes the *éclat* of a "discovery," is often a homage due to the inexhaustible resources and the infinite variety of nature.

II.—On the Development of the Root-cell and its Nucleus in *Chara verticillata* (Roxb.). By H. J. CARTER, Esq., Assistant Surgeon H.C.S., Bombay.

[With a Plate.]

LAST year I found it necessary, on account of the investigations I was then making, to ascertain the physical features which the protoplasm of the first few cells of *Chara* presented on their development from the *nucule*; but, not requiring to go further, I merely commenced from the bursting of the vacuoles of the new protoplasm into each other, and followed this up to the full development of the rotatory motion*. Latterly I have found it

* Ann. and Mag. Nat. Hist. vol. xvii. pp. 110 & 111. pl. 8. fig. 35, 1856.

necessary to extend these researches, that I might ascertain also the changes which the nucleus presents in the freshwater Algæ under cell-division, and having again chosen the roots of *Chara verticillata* for this purpose, I have been led to observe other features in the protoplasm which I had not before noticed, but which, together with the changes exhibited by the nucleus, I will now also describe.

Previously, however, it is advisable that I should state shortly, what has been published respecting the development of the roots of *Chara*, as well as that which is known of the formation of the nucleus generally, in the Vegetable Kingdom.

As regards the former, it has already been stated by C. Müller, in his excellent description of the development of *Chara**, that "as soon as the nucleary membrane [embryo-sac] began to burst through the sporular membrane [brown-coat], like a bladder, and to expand it in a sacciform manner [to form the first cell of the plant-stem], it began to be developed in a sacciform manner on the opposite side" [to form the roots]. Nothing afterwards is mentioned about the roots, saving that "each utricle forms a rootlet, and others follow it from simple vesicular expansion of the nuclear membrane, so that it acquires at this end a complete head of root-fibrils †."

For what is known respecting the formation of the nucleus in the Vegetable Kingdom, I can quote nothing better than the result of Nägeli's researches, which he has summed up in the following manner, viz. :—"The nucleus originates in two ways ; either free in the contents of the cell, or by division of a parent nucleus ‡." The first mode is witnessed in the embryo-sac of the Phanerogamia (*Scilla cernua*, &c.), wherein "globular drops of perfectly homogeneous mucilage with a defined outline" appear ; after which the larger ones present an "enclosed ring ;" and of these, he adds, "there can be no doubt, for the further development also confirms it, that the mucilage-globule is a cell-nucleus, the enclosed ring a nucleolus." He is also of "opinion that the nucleolus originates first, and the nucleus subsequently around it § ;" lastly, he observes, "certain phænomena connect themselves readily with the hypothesis that they [the nucleoli] are utricles ||."

As regards the second mode of origin, viz. that by division, this is witnessed in the nuclei which are formed on each side of

* Ann. and Mag. Nat. Hist. vol. xvii. p. 254, 1846.

† *Idem*, p. 259.

‡ Henfrey's Translations, Ray Soc. Pub. "Reports and Papers on Botany," 1849, p. 168.

§ *Idem*, pp. 106 & 107.

|| *Idem*, p. 172.

the "secondary nucleus in the parent-cell of the spore of *Anthoceros**."

Having thus briefly stated, as far as I am aware, the limits of our knowledge respecting the development of the roots of *Chara*, and the formation of the nucleus of the plant-cell, I will proceed to the subject of this paper, premising a short description of the first root-cell and its contents in the species of *Chara* mentioned, that the reader may recognize without doubt the parts to which I shall have occasion to allude.

This cell is a long narrow cylindrical tube, with one end attached to the *nucule*, and the other free. Its chief elements are the cell-wall and "primordial utricle" of Mohl.

Of the cell-wall nothing more need be said here than that it is as transparent, colourless, and apparently structureless, as unstained glass; but the protoplasm is composed of many organs, which I will first enumerate and then describe in detail. Thus, it is itself surrounded by a cell which we shall call the "protoplasmic sac;" then the protoplasm is divided into a fixed and rotatory portion; these again respectively enclose the nucleus, "granules," and axial fluid; while those small portions of matter which I have before designated as "irregularly shaped bodies†" are common to both.

Protoplasmic Sac.—This sac I have only been able to demonstrate satisfactorily by the aid of iodine and acids applied to the fixed protoplasm when it is about to undergo division for the second root-cell, in the way which will be hereafter mentioned. Its existence, however, might be inferred, from iodine and acids failing to produce any separation between the fixed and rotatory portions of the protoplasm; for these cannot be considered to be in direct union, and therefore, unless supported in their relative position by a membranous sac common to both, would most probably present a line of separation under contraction. Again, the "primary" nucleus ultimately becomes stationary in the midst of the rotating protoplasm, and it also must be fixed to something which is not only stationary itself, but is also carried inwards with this part of the rotating protoplasm, when the latter is condensed and made to leave the cell-wall by acids; for the nucleus, or what remains of it, is at such times seen to be enclosed in the general mass of contracted cell-contents. Lastly,

* *Idem*, 1846, pp. 261 & 262.—*Himantidium pectinale* (Kg.) affords a good example of it among the Algæ; and in the same way I have seen it in some of the cells projecting into the gum-cavities of the bark of *Hyperanthera Moringa* (Roxb.), the *Horse-radish tree* of India; that is, both halves of the nucleus remaining opposite each other, on either side the septum, after the latter has divided the old, from the new cell.

† *Ann. and Mag. Nat. Hist.* vol. xvii. p. 106, 1856.

when the first root-cell assumes part of the function of the cell of the plant-stem, which is frequently the case, the green, peripheral cells appear in an abortive form, disposed in broken scattered lines along its inner surface, and they also are drawn inwards with the general mass of rotating protoplasm under contraction from acids,—with the remains of the nucleus within them again. Now these cells can hardly be supposed to be supported in their position by mere attachment to the cell-wall in the root-cell, any more than they are in the cell of the plant-stem, where they form a distinct layer. Hence, if the protoplasmic sac had not been seen, its existence might thus have been fairly inferred.

Protoplasm.—The protoplasm is a molecular mucus, which, as before stated, is divided into two portions, viz. a fixed and a rotatory portion. The fixed portion occupies the extremity of the cell, and extends backwards for about a hundredth part of an inch (Pl. III. fig. 2 *b, b*), while the rotating portion occupies all the rest of the interior of the tube (*c*). The latter, which is more attenuated than the former, merely encloses the axial fluid, and presents a few of the “irregularly shaped bodies” scattered through its substance, with, perhaps, a globular cell or two in its cavity; but the fixed protoplasm not only also contains a few of these “bodies,” but, in addition, the nucleus, and the group of corpuscles at the extremity of the cell, which I have called “granules.”

Nucleus.—This organ is at first located in that end of the fixed protoplasm which joins the rotatory part (fig. 2 *d*), and then consists of three elements: viz. a transparent, globular cell, which Nägeli has called the “nuclear utricle” (*a*); a more or less transparent mucus, which partly occupies its interior (*b*); and the nucleolus or kernel, which is a spherical body composed of an opaque, yellowish, homogeneous substance, with a single hyaline vacuole in its centre (*c, d*). This is the primary form of the nucleus in *Chara*. Afterwards it enlarges, the transparent portion or cell becomes elliptical, the nucleolus becomes flattened, its single hyaline vacuole is replaced by several which vary in size as well as in number (fig. 3); and these again disappear and reappear, but whether from collapse of the vacuole or change in position of the substance of the nucleolus, I am ignorant. The nucleolus is also now continually but imperceptibly varying its shape, being at one time elongated, and at another subrotund. Finally, when the nucleus has ceased to subdivide for the purpose of furnishing the new cells with nuclei, it moves backwards a short distance, and then becomes permanently fixed to the protoplasmic sac (fig. 4 *d*), where it grows still larger, and, ultimately, its nucleolus divides up into a number of small

nucleoli (fig. 5 *d*). When the second or following root-cell becomes terminal, that is, ceases to throw out any more cells, the nucleus, after the breaking down of the fixed protoplasm, moves about for some time before it becomes fixed; and this is effected partly by the rotating protoplasm and partly by its own locomotive power, which at this time is particularly evident, from gradual change of form while under observation. I have stated that the "nuclear utricle" and its contents are transparent, but this is only in comparison with the turbid fixed protoplasm in which it is imbedded; for when it gets into the clearer cavity of the rotating protoplasm, it not only presents a cloudiness interiorly, but, a certain time after it has become stationary, also becomes filled with vacuoles (fig. 12 *a*), like those which will be found to be developed in the fixed protoplasm preparatory to its assuming a more attenuated form and mingling with the rotatory part. In short, this is the last vital phenomenon presented by this organ; after which it passes into an effete amorphous piece of tissue like cellulose (fig. 6 *d*).

Round or "irregularly shaped bodies."—These are small opaque yellowish masses of protoplasmic (?) matter (fig. 2 *e, e, e*), irregularly scattered throughout both the fixed and rotating protoplasm, and seem to be the same as those which I have described under this head in giving an account of the contents of the protoplasm of the internode of the plant-stem, but they never grow large enough to arrive at those fantastic shapes which are found in the latter*. Like these, also, they are frequently seen appended to, or in the wall of a globular mucus-cell (fig. 14 *a, b*); and this cell may be transparent or clouded by the presence of molecular mucus, while it also frequently manifests a power of movement. Many of these bodies have very much the appearance of the small nucleoli into which the primary nucleolus divides, but as they appear in the cell before the latter takes place, this cannot be their origin.

Granules.—Lastly we come to the granules (fig. 2 *f, &c.*), which are of much interest, on account of their being grouped together in one part of the cell only, their marked characters, and their incessant oscillatory motion. They are situated in the fixed protoplasm close to the free extremity of the cell-wall, and are recognized by their dark margins, greenish colour, constant motion, and tendency to keep together in a group. At first they are round or elliptical (fig. 15 *a*), and of the tint mentioned, but after a while they become subrotund or angular, and colourless (*b*)—apparently effete. When the fixed protoplasm begins to be broken up by the development of vacuoles, they are seen to be scattered

* Ann. and Mag. Nat. Hist. *loc. cit.* p. 106, &c., pl. 8. figs. 11–13.
Ann. & Mag. N. Hist. Ser. 2. Vol. xix.

among the latter ; but after the rotatory movement is completely established, they are no longer to be recognized. Their office seems to be connected with the extension of the cell, as they are only found at its extremity and are in constant motion, but whether this motion is produced by themselves or by the protoplasm in which they are imbedded, I am ignorant. They are also present in the young cell of the plant-stem, but disappear in the way which I have stated, and are then followed by the appearance of the rudiments of the green cells or chlorophyll-bearing cellulæ. Is their office of a like nature, or are they homologous with the latter ?

Vacuoles.—These form no part of the permanent contents of the protoplasm, but are hyaline spaces, which are temporarily developed in the new or fixed protoplasm preparatory to its becoming attenuated and rotatory (figs. 3 *i* & 5 *m*). As they increase in number and size so they burst into each other, until a large space is thus produced in the centre, round which the protoplasm gradually begins to rotate (fig. 6 *t*). As before stated, they appear in the nucleolus and in the mucus-substance of the nuclear utricle when the nucleus becomes stationary ; they also make their appearance in the rotating protoplasm, just preceding its death.

Having now described one of the first root-cells specially and typically, let us turn our attention to the whole bunch as they appear about twenty-four hours after the germination of the *nucule*. Here we shall find, as Müller has stated, that they are developed from the “nucleary membrane” (fig. 1 *h, h'*) (which, for convenience of description, we will now term “embryo-sac”), on one side the plant-stem,—at first as one hemispherical cell, which afterwards divides into four or more root-buds (*f*). The first cell of the plant-stem (*b*), on the other hand, projects from the extremity of the embryo-sac in the form of a parabola, whose summit (*d*) becomes cut off, for the second cell or internode (*c*), by a transverse diaphragm (*i*) ; round the circumference of which, again, on the *lower* side (*e, e*), there is an annular projection, which lodges the protoplasm, that afterwards becomes cut off and divided up into cells for the first node or verticil ; thus allowing direct endosmosis to take place, not only between the first and second cells of the plant-stem, but also between the first cell and the cells of the first node. Sometimes these cells pass into roots, as the cells of any future node may do if the occasion requires it. The figure of the germinating *nucule* which I formerly gave, would have been more complete had I drawn another diaphragm close to the summit of the *nucule*, and placed the roots in a bunch on one side of it, but the reader can do this for himself, and then he will have the first cell of the plant-stem

and first bunch of roots now described*. When a second plant-stem is formed, which is not unfrequently the case, this is developed out of one of the root-buds; hence it is not uncommon to see one of the latter in an intermediate state.

Now if we take the simple root-cell (fig. 2) about the eighteenth hour after germination, when it will be about half an inch long and $\frac{1}{800}$ of an inch broad, and place it in water between two slips of glass for microscopic observation, under a magnifying power of about 400 diameters, we shall find, if the circulation be active and the cell-wall strong and healthy, that the extremity of the latter, together with the nucleus and fixed protoplasm, which, as before stated, is about a hundredth part of an inch long and $\frac{1}{800}$ broad, will, in the course of about twenty-four hours, present the following changes:—

1st stage.—The nucleus, now about $\frac{1}{800}$ of an inch in diameter, is situated in that part of the fixed protoplasm which is next the rotating one; it is also now globular, and its nucleolus, which is about the $\frac{1}{1800}$ of an inch in diameter, spherical and opaque, with the exception of the single hyaline vacuole in the centre. After this, the nucleolus becomes somewhat flattened, its outline becomes subcircular, and it presents several hyaline vacuoles of different sizes. The “granules” are now also in active motion at the other end of the fixed protoplasm, close to the extremity of the cell-wall, but, beyond these and the “irregularly shaped bodies,” the fixed protoplasm presents nothing to interrupt its uniformity throughout its whole extent.

2nd stage.—After a certain time, during which the nucleolus has been successively changing its shape from a subrotund to an elongated form, and *vice versa*, it assumes a grumous appearance, becomes slightly enlarged, and growing fainter in its outline, gradually but entirely disappears, leaving a white space corresponding to its capsule or cell-wall, with a faint remnant of some structure in the centre. Subsequently this space becomes filled up with the fixed protoplasm, and after about an hour and a half, (but this varies,) the nucleus reappears a little behind its former situation, but now reduced in size, and with its nucleolus *double*, instead of single as before (fig. 9); each nucleolus being about one-fourth part as large as the old nucleolus, and hardly perceptible. Meanwhile a faint septum (fig. 3 *g*) is seen obliquely extending across the fixed protoplasm, a little beyond the nucleus; and, if iodine be applied at this time, the division is seen to be confined to the protoplasm, as the latter, from contraction, withdraws itself from each side of the line where the septum appeared, and

* Ann. and Mag. Nat. Hist. *loc. cit.* p. 106, &c., pl. 8. fig. 35.

leaves a free space which is bounded laterally by an uninterrupted continuation of the protoplasmic sac. Hence the demonstration of the existence of this sac to which I have alluded. At this moment a spot, slightly lighter than the rest of the protoplasm, makes its appearance a little beyond the septal line towards the free extremity of the cell, and this is soon followed by the faint appearance of something else in its centre, which, as both become more defined, proves the former to be a new nucleus, and the latter, its nucleolus (*k*). We shall, therefore, henceforth designate the first by the name of "primary," and the second by that of "secondary" nucleus. As the secondary nucleus becomes more evident, its nucleolus also is found to be *double*, and composed of two spherical nucleoli about the same size as those of the primary nucleus, when the latter first returned into view. These nucleoli, like those of the primary nucleus, also become opaque and yellowish, and each presents a single hyaline vacuole or circular area in its centre—sometimes more than one.

The nucleoli of the primary nucleus, after they have become distinct, soon unite (fig. 10) and form one spherical nucleolus (fig. 3 *k*), with a single hyaline vacuole in its centre, thus assuming the form which it first presents when the root has just budded forth from the root-cell of the embryo-sac (fig. 2 *d*). After this the nucleoli of the secondary nucleus also unite in the same way, and present the same spherical form when conjoined.

The primary nucleolus now becomes more opaque, subrotund or elongated, and presents a number of vacuoles of different sizes (fig. 3 *d*); while the septum has become fully formed and has assumed a sigmoid shape (*g*). Thus the second root-cell is completely cut off from the first.

3rd stage.—In this, the cell for the first bunch of rootlets is formed and provided with a nucleus; it commences in a convex, lateral projection of the first root-cell opposite the oblique sigmoid septum (fig. 3 *h*). The primary nucleus now disappears again and undergoes precisely the same changes as those which it did for providing the nucleus for the second root-cell, but its counterpart now appears in the protoplasm occupying the lateral projection (fig. 4 *l*), which also presents a faint septum (*i*) dividing it from the remaining part of the fixed protoplasm, in which the primary nucleus is still imbedded.

As the third nucleus, or that of the lateral cell, becomes more defined, and its nucleoli unite together in the way just described, the septum becomes more evident, and at length we have the lateral cell completely cut off from the first root-cell, and provided with its nucleus (*h*).

Vacuoles now appear in the fixed protoplasm surrounding the primary nucleus preparatory to its being broken down into the rotatory form (fig. 3 *i*).

4th stage.—Here the fixed protoplasm surrounding the primary nucleus becomes entirely broken down by the vacuoles, and the whole of it, blending with the adjoining rotatory portion, now flows freely with the latter, over the septum both of the second root and lateral or rootlet-cells (fig. 4 *k*). While this has been taking place, the primary nucleus has moved a little backwards, and has become permanently fixed to the protoplasmic sac, where the cell-wall has also become elliptically dilated, apparently to receive it (*d, d'*). Vacuoles make their appearance in the midst of the protoplasm of the second root-cell near its septum (fig. 5 *m*); while this cell, now elongated, also gets a twist to one side, from the increased development of the rootlet-cell.

The nucleus in the lateral cell now disappears and returns in the way before mentioned, viz. in two parts, each containing two nucleoli. These undergo the same changes as those before described, ending in a conjunction of the nucleoli of each nucleus (*o, o*). Meanwhile a longitudinal septum has become developed in the lateral cell (*n*), which is thus divided into two, respectively provided with nuclei.

The second root-cell has become more elongated, and the vacuoles have worked a cavity in it, round which the protoplasm is slowly rotating (fig. 6 *t*).

The lateral cell has become divided again by a transverse septum (*s*), which has been accompanied by a division of the nucleus and quadrisection of the nucleolus as before stated; so that there are now four divisions in the lateral cell, each of which presents a single nucleus with a single nucleolus, respectively formed in the way mentioned (*q, q, q, q*).

5th stage.—The second root-cell has reached the state of the first previous to the appearance of the secondary nucleus (fig. 6 *a*); each of the four lateral cells has become elongated, but in different degrees, as in the case of the roots developed from the root-cell of the embryo-sac; one or two in the latter are generally much longer than the others. Meanwhile the primary nucleus has become enlarged, has presented the vacuoles in its mucus-contents to which I have alluded, and its nucleolus has become divided up into a number of small opaque nucleoli (fig. 5 *d*). These disappear and leave the old nucleus in the form of a flat, elliptical, structureless, effete piece of cellulose (?) (fig. 6 *d*); or the nucleus becomes prolonged backwards in the form of a long cell, and the small nucleoli drawn out with it into different shapes and lengths (fig. 13 *a, a, a*). What becomes of the small

nucleoli into which the nucleolus divides, I am ignorant ; whether they become absorbed, or whether they escape from the nucleus into the rotating protoplasm. Judging from what takes place in the cell of the plant-stem, the latter would seem to be their destination ; but, whatever it may be, all trace of them ultimately disappears in the remaining portion of the nucleus.

Thus far, then, we have seen, that the second root-cell and rootlet-cell developed from the first root-cell correspond, in development, with the first cell of the plant-stem and root-cell of the embryo-sac. Moreover, when the second root-cell is prolonged, it undergoes the same changes as the first cell, by which repetition we seem to get further confirmation of what goes on in the embryo-sac before germination becomes evident. All that takes place previous to this, however, is completely shut out from us by the black, opaque colour of the middle coat of the *nucule*, which causes the early part of germination to be as invisible as the glassy transparency of the cell-wall of the root renders its development apparent. Hence, for all that occurs antecedently, we must be guided by inference, and for views on this part of the subject, I can refer the reader to no higher authority than A. Braun, whose observations on the "Nucleus of the Characeæ" are among the papers which have been so happily selected and translated for the advancement of botanical science by Prof. A. Henfrey*. I would here, however, casually notice, that the embryo-sac which turns blue under the action of iodine and sulphuric acid before germination, ceases to do so after the latter has commenced.

It may now be asked, What becomes of the nucleus when it disappears ? In reply to which I can state no more than I have already done,—viz. that all it leaves behind is a clear space, corresponding to the form and size of its capsule or cell-wall, with some faint amorphous tissue in the centre, and that this space also soon becomes obliterated or filled up by the fixed protoplasm, after which no trace of the nucleus remains. Its coming into sight again, with its counterpart too, is so faint, that it seems almost hopeless to endeavour to trace the changes between its disappearing and reappearing again,—reduced in size and with double nucleoli, as I have before stated. But this is certain, that one part moves towards the free end of the root-cell, viz. the secondary nucleus, and the other part, viz. the primary part, retires from it, while the septum is formed between the two in the lighter space of the fixed protoplasm originally occupied by the primary nucleus before its disappearance. It is also worthy of remark, that the part intended for the primary nucleus generally

* Ann. and Mag. Nat. Hist. vol. xii. p. 297, 1853.

appears first, and its nucleoli unite together long before those of the secondary nucleus; while, although the opposite sometimes takes place, it is rare, for I have only observed it twice.

As regards the influence of the nucleus upon the development of the new cells, it will now be evident that, if there be any, it must be derived in the first instance from the parent nucleus, for both the extremity of the second root-cell and the projection for the lateral or rootlet-cell take place before the disappearance of the primary nucleus for providing each of these parts with a new nucleus. But as soon as a trace of the septa respectively cutting off these cells from the remaining portion of the fixed protoplasm, and, therefore, from the old or first root-cell, is visible, the new nuclei respectively also appear in their proper situations; after which the further development of the nuclei and septa progresses *pari passu*. Thus the new cells are never entirely without a nucleus, which would thus appear to exert some influence, directly or indirectly, upon their development, for as soon as the *only* two new cells which the root-cell gives off are formed, the old nucleus becomes effete. At the same time, the general functions of the cell do not depend on the nucleus, for the cell grows larger and the circulation of the rotatory protoplasm continues for an indefinite period after it has ceased to exist; the latter apparently with even greater activity than when it was in full operation. Whether a new cell-bud can originate a new nucleus for itself, or go on growing to the extent of a nucleated cell without a nucleus, I am ignorant. But I am inclined to the opinion that it can do neither, and, therefore, opposed to the view I formerly expressed, when I knew less about the development of the roots of *Chara*, viz. that the root-cells of *Chara*, like the gemmule-buds on the body of *Vorticella*, might be developed "independently of the cell-nucleus*." I should hesitate, therefore, to assert now, that we might state this with certainty even respecting *Vorticella*.

Why the nucleolus should quadruplicate, while the capsule or "nuclear utricle" only (?) duplicates, and, when the division of the latter has been completed, the two nucleoli in each half should unite again into single nucleoli, I am also ignorant. That the nucleus in *Chara verticillata* does invariably undergo this process in the providing of nuclei for new cells, several single and several repeated serial sets of observations on different root-cells enable me to assert. So evident is this, that on one occasion the nucleoli of the secondary nucleus remained separate for five hours, during which they not only constantly changed their position, but grew larger, so that I thought they would

* Ann. and Mag. Nat. Hist. vol. xviii. p. 237, 1856.

never unite, and therefore watched for the time of their provisioning the *third* root-cell; when at the end of the fifth hour union commenced, and an hour afterwards was complete. Twelve hours after, this nucleolus also disappeared; and about an hour and a half from this time, the oblique septum dividing the second from the third root-cell was just visible, with the parent nucleus and its counterpart on each side of it respectively. Can the conjugation of the nucleoli, if it may be so termed, have anything to do with the reproduction or restoration of the size of the nucleus, as in some species of *Spirogyra* and *Diatomea*, where the contents of two cells, which have been derived from an interseptal division of one, unite again to form the spore? The same kind of quadruplication of the nucleolus appears to take place in the formation of the plant-cell of *Chara*, judging from two instances which occurred to me; and in looking for this generally it may be remembered that, whenever double nucleoli are seen in the nuclear utricle of *Chara*, it is a sure sign of this process having taken place, for even if the parent nucleus is ever in such a condition, it is at that time invisible.

On one occasion I found two nuclei with their two nucleoli respectively still disunited, in the axial fluid of the rotating protoplasm, while each nucleus was reduced to a clear transparent oblong cell; and on looking for the primary nucleus in its natural position, as well as for the nucleus of the rootlet-cell, the projection for which was already somewhat advanced, I found that they were both absent, while the presence of vacuoles in the protoplasm filling the projection for the rootlet-cell, which was not yet cut off from the parent, showed not only that this protoplasm was undergoing solution, but, also, that the development of the rootlet-cell had been arrested. No doubt, therefore, remained in my mind that the two nuclei in the axial fluid were the primary nucleus and the nucleus of the rootlet-cell. In these instances the nucleoli were clearly seen, and they presented the form of spheres filled or lined with a semi-opaque, homogeneous, yellowish substance, in the centre of which, on the surface of each, was a circular hyaline area or vacuole. In the nucleus nearest the free end of the root-cell, whose nucleoli were separated for some distance from each other (fig. 11), a transparent cell round each nucleolus could be perceived (*a, a*), but this was not apparent in the nucleus which was furthest from the end of the root-cell, whose nucleoli were in contact. The position of these nuclei, away from their proper situations, does not seem inexplicable, when we remember the migrating power of this organ, the want of a septum to keep the nucleus of the root-cell in its place, and the vacuolar solution that the fixed protoplasm was undergoing in which they ought to have been

imbedded; nor can the approximated state of the nucleoli in one nucleus and their separation in the other, coupled with their relative position in the cavity of the rotating protoplasm, fail to point out which was intended for the primary nucleus, and which for that of the rootlet-cell. Thus these nuclei, being in the clear cavity of the axial fluid instead of in the fixed protoplasm, afforded a much better view of the condition they and their nucleoli would probably have been in, had they remained in their natural situations; and the duality of the nucleolus indicating a recent division of the mother nucleus, while the second cell had been provided, left, with what has been before stated, no doubt in my mind, that this must have been the second division of the primary nucleus for provisioning the rootlet-cell.

It is by no means uncommon, moreover, to find the nucleolus of the primary nucleus elongate and irregularly subdentate at the border, and half an hour afterwards to find it subrotund, and so on to change from elliptical to subrotund successively for several times, as before stated. I have also mentioned the appearance and disappearance of the vacuoles in it, which Nägeli calls "froth" (*loc. cit.*); and the evidence of locomotive power in the nucleus itself, or in what Nägeli calls the "nuclear utricule." I have, however, never seen any granular matter in it, neither have I ever seen any granular matter in the mucus-contents of the nucleus with the microscopic power mentioned; but, like the nucleolus, it presents vacuoles, though this is only preparatory to becoming effete. The nucleolus sometimes presents a grumous appearance, as before stated, but this is, generally, just before it disappears, and I am not certain whether it does not depend on an increase in the number of vacuoles.

Iodine makes the nucleolus contract and assume a deep brown-red colour, which yields to water. Sulphuric acid causes it to swell up and disappear instantly, leaving nothing but the nuclear utricule behind, unaffected; just as when the nucleolus disappears preparatory to the formation of a new cell. If, however, a weak solution of iodine be first added, so as only to contract the nucleolus slightly, the sulphuric acid does not act so rapidly, and then it may be seen to expand under the eye until its outline alone remains visible, with the vacuoles, which do not disappear under these circumstances. I have never been able to demonstrate a capsule round the nucleolus *in situ*, whether young or old, double or single, though I have tried in various ways to do so, from the deceptive appearance which it frequently presents of having one; nevertheless, in the case mentioned where the nucleus was not *in situ*, a transparent capsule did appear to exist round each nucleolus. When the nucleolus be-

comes invisible, or very faint under the action of sulphuric acid, iodine fails to restore its form, or render it more distinct; and in no instance have I ever been able to produce the characteristic blue colour of starch in any part of the nucleus.

We now come to the offices of the nucleus, of which nothing more is revealed to us in the development of the roots of *Chara*, than that, so long as new cells are to be budded forth from the one to which the nucleus belongs, the nucleus continues in active operation, but when this ceases it becomes effete; while the rotation of the protoplasm and subsequent enlargement of the cell, &c., which are much better exemplified in the plant-stem than in the root-cell, go on after the nucleus ceases to exist. Hence the development of the root-cells of *Chara* affords us nothing positive respecting the functions of this organ; and, therefore, if we wish to assign to it any uses in particular, they must be derived from analogy with some other organism in which there is a similar nucleus whose office is known. Now, if for this purpose we may be allowed to compare the nucleus of *Chara* with that of the Rhizopodous cell which inhabits its protoplasm, we shall find the two identical in elementary composition; that is, both consist at first of a "nuclear utricule," respectively enclosing a structureless, homogeneous nucleolus; the latter, too, in both, is endowed with a low degree of movement. After this, however, the nucleolus of the *Rhizopod* cell becomes granular and opaque; and, when, under circumstances favourable for propagation, a new cell-wall is formed around the nuclear utricule,—or this may be an enlargement of the nuclear utricule itself,—I do not know which; the granular substance of the nucleolus becomes circumscribed, and shows that it is surrounded by a spherical, capsular cell; the granules enlarge, separate, pass through the spherical capsule into the cavity of the "nuclear utricule;" a mass of protoplasm makes its appearance, and this divides up into monads, or, as I first called them, "gonidia*." The nucleolus of *Chara*, on the other hand, after having provided the two cells developed from its own root-cell, becomes stationary, and also divides up into a number of small, round, graniform nucleoli, which disappear in some way or other unknown to me, leaving the nuclear utricule, at least, effete. Whether these small nucleoli are ultimately dissolved, or find their way into the rotating protoplasm, I am, as I have before stated, ignorant; but, so far as this multiple division goes, we have an analogous termination between the nuclei of these two organisms; and when we remember that the nucleus of the cell in which the *globule* of *Chara* originates, must furnish all the cells with nuclei which bear respectively the antherozoids,—that these nuclei are very

* Ann. and Mag. Nat. Hist. vol. xvii. p. 101, 1856.

small, so small indeed that they are but granules in size, compared with the nuclei of the plant and root-cells,—it does not seem far-fetched to assume that the nucleus is an organ of generation.

Further, should it hereafter be proved that the rhizopodous cells are developments of *Chara* itself, and not a foreign organism, it might not be found difficult to trace a connexion between the so-called "gonidia" and the "spiral filaments." Thus *Chara*, in some forms, would then be an animal, and in others a vegetable, according to the distinction between it and *Amœba*, which will presently be mentioned; for the rhizopodous cells do not produce the "gonidia" or monads until they have enclosed a portion of the cell-contents, after the manner of *Amœba* when taking its food. Again, I have already shown how the nucleus of the latter divides up into granules and cells producing new beings, and how it becomes lost in the development of the ovules*, and Stein has shown that the nucleus of *Vorticella* becomes divided up into cells to produce a new litter; also, that it shrinks into a small elliptical effete mass of fine granules in the development of *Acinetæ* through the *Acineta*-form, which I have frequently been able to confirm. So that, if the nucleus in *Amœba* and *Vorticella* be identical with that of *Chara*, we shall probably not be far wrong in assigning a generative power to it generally; that is, through duplication in common reproduction and by multiple division in the true process of generation. We must therefore, if we adopt these views, regard the nucleus of the *globule* as merely a modification of that of the cells of *Chara* generally, to meet the requirements of the case; and hence as a subordinate organ, which, together with the other parts of the protoplasm, is subject to a common developmental power. It has already been stated, that the nucleus perishes as soon as its functions cease, while the cell to which it belonged goes on growing. Thus the internode of the large *Nitella* of Bombay†, which may be half a foot long, loses its nucleus, probably when, as a cell, it does not exceed the 100th part of an inch, for the nucleus disappears long before the layer of green-cells is formed.

It has not, however, been shown what becomes of the small nucleoli of the effete nucleus; and perhaps it would be as well, not to assume that no more new cells can be formed after this takes place; for, if the cortical layer of cells is ever added to the first internodes and branches of the young plant of *Chara verticillata*, which I have already stated to commence in the simple form of *Nitella*, it must be some time after the nucleus has ceased to

* Ann. and Mag. Nat. Hist. vol. xviii. p. 222.

† *Idem*, vol. xvii. p. 102, foot-note.

appear in its ordinary form or as a whole; for at present I have a dozen plants with the *nucules* attached to them respectively, and each plant about one-third of an inch in length, without the least appearance of cortical cells, although each is composed of three or four internodes and several branches; if the cortical cells appear hereafter, they may, perhaps, be formed like the other cells, viz. by projections of the mother cell-wall, in the form of grooves, which, lodging a portion of protoplasm, are ultimately cut off from the parent cell or internode; in which case they must be provided with nuclei from the remnants of the old nucleolus, or nuclei altogether *de novo*.

While the component parts of the first cell of the root of *Chara* are still fresh in the mind of the reader, it seems advisable that they should be compared with those of *Amæba*. *Chara* lives by nutriment obtained through endosmosis; *Amæba*, by taking in the crude material direct, and, having abstracted the nutritious parts by the process of digestion, ultimately throwing off the refuse. *Chara* is a vegetable, though there are animal cells which also live by endosmose; but *Amæba* cannot be a vegetable, if we admit the distinction that I have given, viz. the taking in of crude material. Nevertheless the root-cell of *Chara* and *Amæba* greatly resemble each other.

Thus the cell-wall of the former corresponds with the pellicular secretion or capsule of *Amæba*, which, in *Arcella*, &c., appears as a shell. The protoplasmic sac may correspond with the pellicula itself and diaphane. The nucleus is identical, and situated in the fixed portion of the protoplasm, as it appears in the fixed molecular sarcode of *Amæba*, when the latter assumes a spherical form. [In my Notes on the Organization of the Infusoria*, I have called the "nuclear utricule" the "capsule," and the "nucleolus" the "nucleus."] The "granules" of the fixed protoplasm have exactly the same greenish tint and appearance that the "granules" of the sarcode in *Amæba* present, and the former appear to be vicarious in function, if not homologous with the green-cell of the plant-stem; that is, when the former make their appearance, the latter disappear. The rotating protoplasm corresponds with the internal mucus of *Amæba*, to which I would confine the term "sarcodet," and the vacuoles with the vesicula and vacuoles of the substance of *Amæba*; hence it would appear that, as a cavity is formed in the protoplasm of the cell of *Chara* by the bursting of the vacuoles into each other, round which the rotating protoplasm turns, so it may be the vesicula which thus becomes distended in *Amæba* to render it spherical, and hence the appearance of the fixed sarcode on the

* Ann. and Mag. Nat. Hist. vol. xvii. p. 356.

† *Idem*. See the definition of these terms.

side of it in which the nucleus is imbedded. That the vesicula, when greatly distended, does render some of the Infusoria spherical, may easily be seen by the state in which *Plasconia* and *Vorticella* burst from their cysts respectively when the vesicula is expanded to the utmost to produce the rent, and then subsiding after the animalcule has effected its escape, thus allows of its returning to its natural form; and it is not unreasonable to infer that the same thing takes place in *Amæba*, to render its plane form spherical, and *vice versâ**. Nor should we omit in this analogy the vacuolation which takes place in the protoplasm of *Chara* just before the cell dies, or when it is weakened by disease or injury, which is a common occurrence in the vesicula and its vacuolar system in *Amæba* and other Infusoria under similar circumstances.

The most interesting point, however, which this analogy brings forth is the correspondence between the rotatory motion of the protoplasm in the cell of *Chara*, and that of the sarcode of *Amæba* and other Infusoria; since, by considering this motion in different organisms, we may perhaps arrive at some notion of the cause by which it is produced in all. In the Planariæ and Rotatoria, the lash of cilia, which projects from the hepatic cells that line the stomachs of these animalcules respectively, appears to rotate the food during the process of digestion; but in the second part of the alimentary canal of the Rotatoria, where there are no hepatic cells, the surface is seen, on the approach of anything into it, to be covered with cilia. Again, in *Vorticella* and *Paramecium Aurelia*, the digestive globules also are slowly circulated round the abdominal cavity, if I may so term it, in the midst of the sarcode or internal mucus; and when we watch this circulation narrowly, for instance, in the posterior part of *Vaginicola crystallina* (Ehr.), we see that the bodies in which the chief motion exists are very minute and apparently stationary, and that, while their movements are very rapid, the circulation of the pellets of food is very slow; hence they would appear to be cilia. The same kind of circulation occurs in *Amæba*, but is so tardy, and this Infusorium is so incessantly changing its shape, that it is not seen, under ordinary circumstances. The movement of the rotating protoplasm in the Characeæ is also very slow; for, when it is viewed in the long internodes of *Nitella* with a very low power, or even with the naked eye, it seems hardly to move faster than the foot of a Gasteropod; still there is no positive evidence that it moves round the cell after the manner of the latter, although it would appear to possess the power of movement *per se*. Hence the question remains undecided, viz. whether it moves round the cell by itself, or by the aid of

* *Idem*. See remarks on this point, vol. xviii. p. 131.

cilia disposed on the inner surface of the protoplasmic sac, in like manner to those which appear to exist in the abdominal cavity of *Vaginicola crystallina*, and which have been seen and drawn by the Hon. and Rev. S. G. Osborne, and confirmed by Mr. Jabez Hogg, in *Closterium Lunula**.

By the latter observation I do not mean it to be inferred, that I think the backward and forward, &c. motions of the corpuscles of *Closterium*, *Surirella splendida* (Ehr.), and *Spirogyra*, are, altogether, thus produced; for in the two latter organisms they seem to be borne along in or upon minute mucus-threads which creep over the internal surface of the primordial utricle, or stretch across to the suspended nucleus. In the terminal cells of the filaments of *Spirogyra*, especially, the advancing point of these threads may often be seen, as well as the end of the line of molecules trailing after them; and several such threads may also be seen in motion *en masse*, indicative of the whole of the internal part of the primordial utricle being composed of them, unless they form a particular structure of themselves. It has lately struck me too, while watching *Surirella splendida*, that the motion which is seen in the corpuscles of its interior is precisely similar to that of foreign bodies over its surface, which I have endeavoured to prove by analogy to depend, in the *Diatomeæ* generally, on the presence of a transparent envelope endowed with locomotive and prehensile power †.

It might be said, perhaps, by some, that in the present state of our knowledge, the comparison between a plant and an animal is not allowable; but the answer to this respecting *Amæba* is, that there is nothing on the animal side of this organism that offers for comparison equal to the organisms on its vegetable side, taking it even generally or particularly. Again, it might be said that I was formerly of opinion that the rotating protoplasm circulated round the cell by itself; but I was then not aware of the existence of the protoplasmic sac or a fixed membrane inside the root-cell, on the apparent absence of which this view was chiefly grounded. Lastly, it might be said that I formerly tried to prove that the "gonidia" developed from the Rhizopodous cells of the protoplasm were the offspring of a parasite, and now I have hinted that they may be found to be developments of *Chara* itself. Proof of the latter, however, is very remote; but when we find that there exists an intimate analogy between the nucleus of the cell of *Chara* and that of this Rhizopodous cell, as well as that of *Amæba*, &c., both in form and, probably, office, and that the nucleus of the Rhizopodous cell divides up into granules for the production of the "gonidia"

* Quart. Journ. Microscop. Sci. vol. ii. p. 234, 1854.

† Ann. and Mag. Nat. Hist. vol. xviii.



